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Maize oleosin is correctly targeted to seed oil bodies in *Brassica napus* transformed with the maize oleosin gene

(lipoprotein/napin promoter)

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ABSTRACT **Oleosins are small hydrophobic abundant proteins localized in the oil bodies of plant seeds. An oleosin gene from the monocotyledonous maize (*Zea mays* L.) was transferred into the dicotyledonous *Brassica napus* L. using *Agrobacterium*-mediated transformation. The maize oleosin gene was placed under the control of either its own promoter/terminator or the promoter/terminator of a *Brassica* seed storage protein (napin) gene. Southern blot analyses of individual transformed plants suggested that the oleosin gene from either construct was incorporated into the *Brassica* chromosomes without appreciable structural alterations. The amount of construct incorporated was from 1 to >10 copies per haploid genome, depending on the individual transformant. Maize oleosin mRNA and protein were detected only in the transformants containing the napin gene promoter/terminator constructs; these transformants were studied further. Northern blot analyses of RNA isolated from different tissues and seeds of different developmental stages indicated that the maize oleosin mRNA was present only in the maturing seed. Approximately 1% of the total protein in mature seed was represented by maize oleosin. Subcellular fractionation of the mature seed revealed that 90% or more of the maize oleosin, as well as the *Brassica* oleosin, was localized in the oil bodies. The results show that a monocotyledonous oleosin possesses sufficient targeting information for its proper intracellular transport in a dicotyledon and also suggest that the napin gene promoter/terminator of *Brassica*, or equivalent seed storage protein regulatory elements of other plant species, may be used to express genes for the genetic engineering of seed oils.**

The storage triacylglycerols in seeds are confined to discrete spherical organelles called oil bodies (1–3). Each oil body of 1 μm in diameter contains a matrix of triacylglycerols surrounded by a layer of phospholipid (3) embedded with abundant and unique proteins called oleosins (1). Oleosins are hydrophobic proteins of low molecular mass ranging from 16 to 26 kDa, depending on the plant species. The possible functions of oleosins include maintaining the structural integrity of oil bodies and serving as a recognition signal for lipase binding during oil mobilization in seedlings. The amino acid sequences and the predicted secondary structures of the two major oleosins of 16 and 18 kDa from the monocotyledonous maize have been obtained from their DNA sequences (4, 5). Each oleosin molecule contains a relatively hydrophilic N-terminal domain of ≈48 amino acid residues, a central completely hydrophobic domain of ≈77 amino acid residues, and an amphipathic α-helical domain of ≈33 amino acid residues at or near the C terminus. Although each of the two maize oleosins contains these three structural domains, their amino acid and gene nucleotide sequences are significantly homologous only in the central hydrophobic domains. A subsequent study shows that the oleosin from dicotyledonous carrot somatic embryos also contains the above three distinct structural domains and, again, its amino acid sequence is highly similar to those of the two maize oleosins only in the central hydrophobic domain (6).

Oil bodies are synthesized during seed maturation by the following proposed mechanism (1). Triacylglycerols are synthesized in the endoplasmic reticulum and sequestered between the two phospholipid layers of the membrane at a particular region. A budding vesicle of triacylglycerols surrounded by a layer of phospholipids is then formed, and the mature vesicle is released into the cytosol as an oil body. In maize, the oleosins are synthesized in the rough endoplasmic reticulum with no appreciable co- or post-translational processing (7). The location and exact nature of the signal in the oleosin molecule required for the transport of the newly synthesized protein from the endoplasmic reticulum to the oil body are unknown. It has been proposed that this intracellular transport signal resides in the central domain of the oleosin in view of its long stretch of 77 hydrophobic amino acid residues and its highly conserved amino acid sequences among different oleosins (4).

We have transformed *Brassica napus* (a dicotyledon) with a maize (a monocotyledon) gene encoding an oleosin of 18 kDa (referred to as maize oleosin hereafter). In the transformants containing the maize oleosin gene under the control of its own 5′ and 3′ regulatory elements, mRNA and oleosin were not detectable. However, in the transformants containing the maize oleosin gene under the control of regulatory elements of a *Brassica* gene encoding napin (a major seed storage protein), mRNA and oleosin were produced abundantly. In these latter transformants, the expression of the maize oleosin gene was under temporal and tissue-specific controls similar to those for the napin gene. Also, the maize oleosin was exclusively localized in seed oil bodies, indicating that maize and *Brassica* share highly similar oleosin targeting mechanisms. Herein we report our findings.

MATERIALS AND METHODS

Construction of Binary Vectors Containing the Maize Oleosin Gene. The scheme for the construction of two binary vectors containing the maize oleosin structural gene is shown in Fig. 1.

A 4.0-kilobase (kb) *Hin*dIII fragment of the maize oleosin genomic DNA containing 1.93-kb 5′ untranslated, 0.56-kb translated, and 1.48-kb 3′ untranslated regions was described (4). This *Hin*dIII fragment was cloned into a binary vector pCGN1557 (9) to generate pCGN1869.

In another construct, an *Eco*RI fragment of 1.2 kb, containing the entire maize genomic oleosin coding sequence (563 base pairs) flanked with 5′ untranslated (437 base pairs) region and a 3′ untranslated (180 base pairs) region, was

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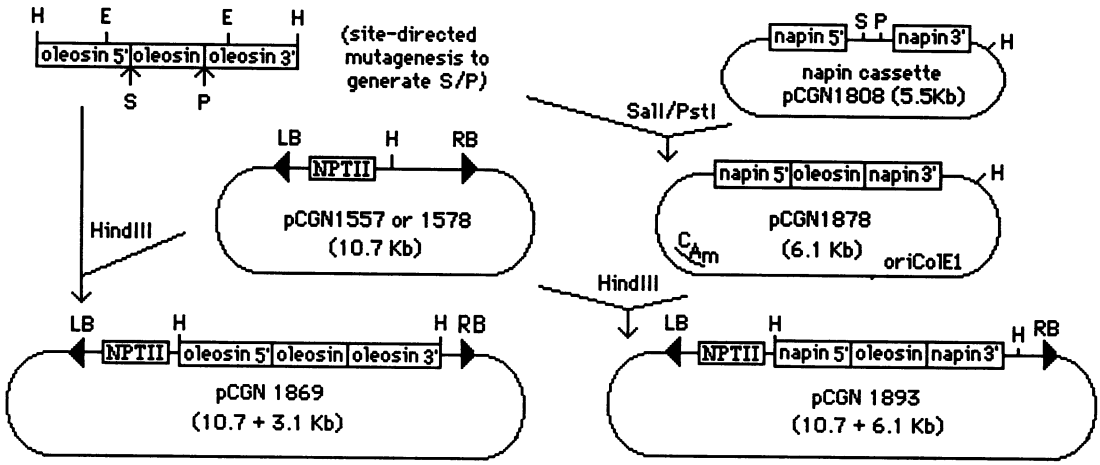

FIG. 1. Construction of a binary vector (pCGN1869) containing a 4.0-kb maize oleosin genomic DNA and another binary vector (pCGN1893) containing 0.56 kb of the complete coding sequence of maize oleosin genomic DNA flanked with a 1.75-kb 5′ promoter region and a 1.25-kb 3′ terminator region from a *B. campestris* napin gene. The 4.0-kb maize oleosin genomic DNA, in M13mp19, was described (4). Details of pCGN1808 containing the 5′ and 3′ flanking sequences of the *Brassica* napin gene will be reported (J.C.K., unpublished data). Details of the binary vectors pCGN1557 (used to generate pCGN1869) and pCGN1578 (used to generate pCGN1893) containing left border and right border of the transferred DNA (T-DNA) of *Agrobacterium* and the plant selectable marker neomycin phosphotransferase driven by the cauliflower mosaic virus 35S promoter have been reported (9). E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sal* I; LB, left border; RB, right border; NPTII, neomycin phosphotransferase II; CAM, chloramphenicol resistance.

subcloned into pBluescript SKII(−) (Stratagene). This plasmid was subjected to site-directed mutagenesis (10) to generate a *Sal* I site at the 5′ end and a *Pst* I site at the 3′ end of the coding sequence. The resulting *Sal* I–*Pst* I fragment was placed between the 5′ and 3′ flanking sequences of the *Brassica campestris* napin gene to generate a chimeric expression unit (pCGN1878). This plasmid was linearized with *Hin*dIII and inserted into a binary vector pCGN1578 (9) to generate pCGN1893.

Transformation of *B. napus*. *Agrobacterium tumefaciens* EHA 101 (kindly provided by Elizabeth Hood, Biology Dept., Utah State Univ., Logan, UT) carrying pCGN1869 or pCGN1893 was used to transform hypocotyl explants from 6-day-old *B. napus* cv. Westar seedlings as described (9). Treated explants were selected on kanamycin (25 mg/liter). Shoots were selected and placed on a rooting medium containing kanamycin (50 mg/liter). Leaves from rooted plants were tested for neomycin phosphotransferase II enzyme activity using the dot-blot assay (9). Those plantlets possessing neomycin phosphotransferase II activity were retained and allowed to grow to maturity in a greenhouse.

DNA Extraction and Southern Blot Analyses. DNA was isolated from leaves by the procedure of Taylor and Powell (8). Isolated DNA was digested with *Hin*dIII, and the resulting fragments were separated by electrophoresis in a 0.7% agarose gel and transferred to a nylon membrane (Zeta-Probe, Bio-Rad). Conditions for prehybridization, hybridization, and washing were as described by the manufacturer. A 0.56-kb *Sal* I–*Pst* I fragment of the maize oleosin coding region (Fig. 1) was ^{32}P-labeled using the random oligonucleotide method (11), and the labeled product was used as probe.

RNA Extraction and Northern Blot Analyses. Total RNA was prepared from leaves, fruit pods, and flower petals of mature plants and from roots of 5-day-old seedlings of the various transformants by the LiCl method (12). RNA was glyoxylated, fractionated by 1% agarose gel electrophoresis, and transferred to a nylon membrane. Prehybridization, hybridization, and washing conditions were essentially as described for Southern blot hybridization.

Oleosin Isolation and Antibody Preparation. Oleosins from the embryos of mature maize (*Zea mays* L., inbreed line Mo 17) kernel and the dehulled seed of *Brassica* (*B. campestris* L., cv. R500) were isolated by a gel-extraction procedure (4, 13). Antibodies against purified oleosin were raised in chickens (13).

SDS/PAGE and Immunoblot Analysis. Proteins in the various subcellular fractions were resolved by SDS/PAGE (4, 13), using 12.5% and 4.75% polyacrylamide gels in the separating gel and stacking gel, respectively. After electrophoresis, the gel was stained with Coomassie blue R-250 and destained. For immunoblot analysis, the proteins in an SDS/PAGE gel were transferred to a nitrocellulose membrane in a Bio-Rad Trans-Blot cell (13). The membrane was incubated with 4-chloro-1-naphthol containing H_2O_2 for color development (13).

Subcellular Fractionation. Dehulled mature seed of transformant BP06 was homogenized at 4°C in 20 ml of grinding medium with a Polytron homogenizer (Brinkmann) fitted with a PTA 10 generator at high speed for 40 s. The grinding medium contained 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$, 2 mM dithiothreitol, and 0.15 M Tricine·KOH (pH 7.5). The homogenate was filtered through a 100 × 100 μm Nitex cloth (Petko, Elmsford, NY). The filtrate (15 ml) was placed at the bottom of a 30-ml centrifuge tube, and 15 ml of flotation medium (grinding medium containing 0.4 M instead of 0.6 M sucrose) was layered on top. The tubes were centrifuged at 10,000 × *g* for 20 min in a swinging bucket rotor. The oil layer on top was collected and resuspended in 15 ml of grinding medium containing 2 M NaCl. The resuspension was placed at the bottom of a 30-ml centrifuge tube; 15 ml of floating medium (grinding medium containing 2 M NaCl and 0.25 M instead of 0.6 M sucrose) was layered on top, and the tube was centrifuged. The oil layer on top was collected and resuspended in 15 ml of normal grinding medium, and 15 ml of normal flotation medium was layered on top. After centrifugation, the oil layer on top was collected and resuspended with grinding medium. In all of the above flotation centrifugations, the supernatants remaining after the oil layers had been removed were collected and centrifuged at 100,000 × *g* for 90 min to yield a 100,000 × *g* supernatant and a 100,000 × *g* pellet.

Maize oleosin used in experiments that tested the possibility of its artificial binding to *Brassica* oil bodies was obtained from isolated maize oil bodies (7). The isolated maize oil body fraction was treated with an equal volume of diethyl ether three times to remove triacylglycerols (7). The remaining suspension of oleosins and phospholipids was either used

directly or subjected to treatment with 2 vol of chloroform/methanol, 2:1 (vol/vol), to remove the phospholipids. The two preparations were used directly or sonicated with a Bronwill Biosonik IV ultrasonic generator fitted with a 3-mm (diameter) probe at 200 speed for three 20-s periods; samples were cooled between sonications in an ice bucket. The maize oleosin preparation and dehulled nontransformed *Brassica* seed, in a proportion similar to that in *Brassica* transformant BP06, were homogenized in the grinding medium. Subcellular fractionation was performed as described above.

RESULTS

Transfer of a Maize Oleosin Gene into *Brassica* Plants. *Agrobacterium*, which carried one of the two binary vectors containing the maize oleosin gene with different 5′ and 3′ regulatory elements (Fig. 1), was used to transform hypocotyl explants of *B. napus*. The vector pCGN1869 contained the maize oleosin gene flanked by its own 5′ and 3′ regulatory elements (referred to as the maize promoter construct). The other binary vector pCGN1893 had the oleosin gene placed between the 5′ and 3′ regulatory elements from a *Brassica* storage protein (napin) gene (referred to as the *Brassica* promoter construct). Two plants transformed with the maize promoter construct and four plants transformed with the *Brassica* promoter construct were examined.

In both groups of transformants, the maize oleosin gene coding sequence with the promoter DNA and terminator DNA was incorporated into the *Brassica* chromosomes with no apparent structural alterations (Fig. 2). In Southern blot analyses of genomic DNA, the expected *Hin*dIII fragments of 4.0 and 6.1 kb (see Fig. 1) were observed in the plants transformed with the maize promoter construct and the *Brassica* promoter construct, respectively (Fig. 2). Plants MP01 and MP03 contained 10 or more copies of the inserted DNA per haploid genome. Plants BP01, BP05, and BP09 had 1 or 2 copies, and BP06 possessed ≈10 copies of the inserted DNA per haploid genome.

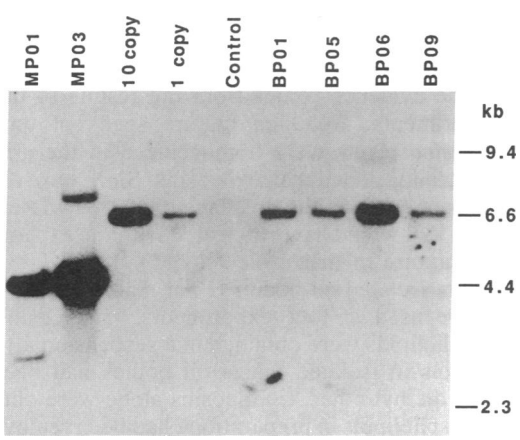

FIG. 2. Southern blot analyses of genomic DNA extracted from leaves of transformed *B. napus* plants. Plants MP01 and -03 were transformed with maize promoter construct (pCGN1869), which contained the maize oleosin coding region and its own maize promoter and terminator. Plants BP01, -05, -06, and -09 were transformed with *Brassica* promoter construct (pCGN1893), which contained the maize oleosin coding region and the *Brassica* napin gene promoter and terminator. A control plant was obtained without *Agrobacterium* treatment. Each lane was loaded with 5 μg of total *Brassica* DNA digested with *Hin*dIII. For copy number reconstruction, unlabeled probe DNA equivalent to 1 or 10 copies transformed DNA per haploid *Brassica* genome was added to 5 μg of *Hin*dIII-digested control DNA. The weight of a haploid *B. napus* genome was assumed to be 1.6 pg (14). The probe was a ^{32}P-labeled 0.56-kb *Sal* I–*Pst* I oleosin gene coding sequence. Sizes of DNA markers are labeled on the right side.

Detection of Maize Oleosin mRNA in Transformed *Brassica* Plants. The transformed *Brassica* plants were analyzed for the presence of maize oleosin mRNA in their seeds by using Northern blots. Preliminary experiments on some transformants indicated that the levels of maize mRNA were highest between 25 and 35 days after pollination. Accordingly, RNAs extracted from the embryos of the transformants 25 (data not shown) and 30 (Fig. 3*A*) days after pollination were analyzed. Only the transformants containing the *Brassica* promoter construct possessed the maize oleosin mRNA. The length of the mRNA was ≈1100 bases, similar to that observed in the maize embryos and that predicted from the genomic sequence (4). Transformant BP06, which possessed more copies of the inserted maize oleosin gene than the other three BP transformants (Fig. 2), contained the highest level of maize oleosin mRNA. In a developmental study of the seed from transformant BP06, the maize oleosin mRNA appeared 18 days after pollination, peaked at day 30, and disappeared completely at day 45 (Fig. 3*B*). Also, in this transformant, the maize oleosin mRNA was present only in the maturing embryos and not in leaves, roots, pods, and petals (Fig. 3*C*). The temporal and tissue-specific expression of the maize oleosin gene is reflective of the regulatory nature of the *B. napin* gene promoter/terminator (15).

Detection and Localization of Maize Oleosin in Transformed *Brassica* Plants. Total proteins extracted from the mature seeds of the various transformants were analyzed by SDS/PAGE. From each sample, many major protein bands were observed (Fig. 4). These major proteins have been described (7, 15) and represent major storage proteins, napin (4 kDa and 13 kDa) and cruciferin (24–30 kDa), and oleosin (20 kDa). The samples from the two plants transformed with the maize promoter construct contained no maize oleosin as monitored by Coomassie blue staining and immunoblot analysis (Fig. 4). The samples from the four plants transformed with the *Brassica* promoter construct possessed an additional protein

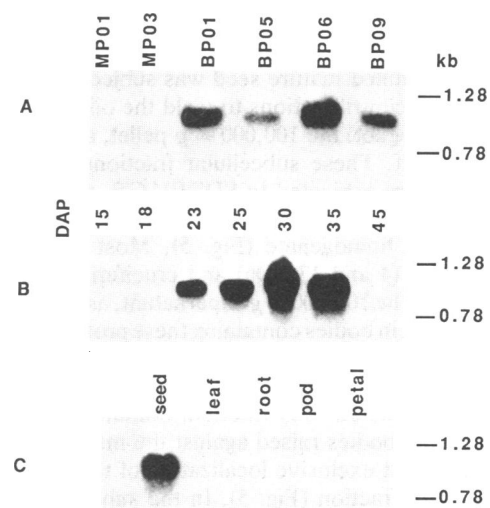

FIG. 3. Northern blot analyses of total RNA extracted from transformed *B. napus* plants. Only the portion of the blots corresponding to the visible hybridized RNA is shown; other portions of the blots did not have positive hybridization. Each lane was loaded with 10 μg of total RNA. The probe was a ^{32}P-labeled 0.56-kb *Sal* I–*Pst* I oleosin coding sequence. (*A*) RNA extracted from seed embryos of the various transformants 30 days after pollination. (*B*) RNA extracted from seed embryos of the transformant BP06 at various days after pollination (DAP). (*C*) RNA extracted from seed embryos 30 days after pollination, leaves, pods, and petals of mature plants, and roots of 5-day-old seedlings of the transformant BP06.

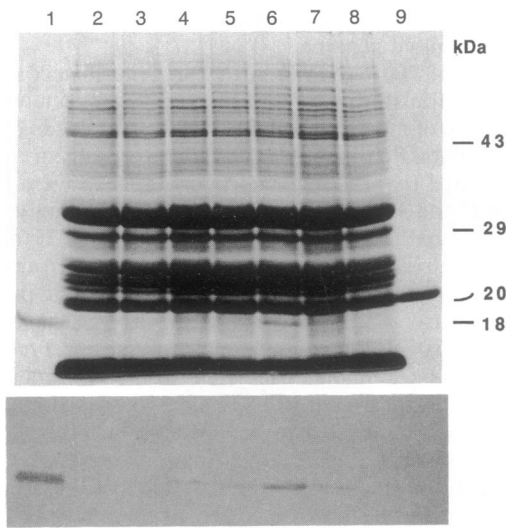

FIG. 4. Separation of seed proteins of various transformed *B. napus* plants by SDS/PAGE and immunoblot analysis of the separated proteins. A control plant was obtained without *Agrobacterium* treatment. The proteins in the gel were either stained with Coomassie blue (*Upper*) or blotted onto a nitrocellulose membrane for immunodetection with antibodies raised against the maize oleosin (*Lower*) (only the portion corresponding to proteins of 10–30 kDa is shown). Isolated maize oleosin (lane 1) and *Brassica* oleosin (lane 9) were used as references. Lanes: 2, MP01; 3, MP03; 4, BP01; 5, BP05; 6, BP06; 7, BP09; 8, control. Molecular masses of proteins are shown on the right.

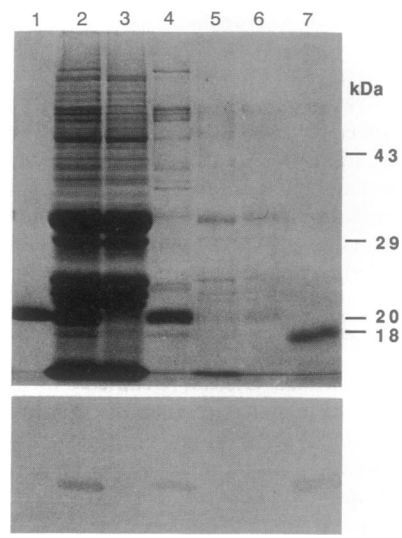

FIG. 5. Separation of seed proteins of the *B. napus* transformant BP06 by SDS/PAGE and immunoblot analysis of the separated proteins. Dehulled mature seed was homogenized, and the total homogenate (lane 2) was subjected to flotation and differential centrifugations to yield an oil body fraction, a 10,000 × *g* pellet, a 100,000 × *g* pellet, and a 100,000 × *g* supernatant (lanes 4, 5, 6, and 3, respectively). The total homogenate and the subcellular fractions were applied, with loading samples adjusted to represent amounts derived from an equal quantity of the homogenate, to different lanes of the gel. The proteins in the gel were either stained with Coomassie blue (*Upper*) or blotted onto a nitrocellulose membrane for immunodetection with antibodies raised against the maize oleosin (*Lower*) (only the portion corresponding to proteins of 10–30 kDa is shown). Isolated maize oleosin (lane 7) and *Brassica* oleosin (lane 1) were used as references. Molecular masses of proteins are shown on the right.

of ≈18 kDa. This additional protein was the maize oleosin as evidenced by its (*i*) comigration with the authentic maize oleosin in the gel, (*ii*) recognition by antibodies raised against the maize oleosin, and (*iii*) absence in the untransformed plant sample. Among the four transformants, BP06 contained the highest level of maize oleosin (Fig. 4). In this transformant, we estimated that the maize oleosin represented ≈1% of the total seed protein.

The maize oleosin in the seed of transformant BP06 was further studied for its subcellular location. The total homogenate of the dehulled mature seed was subjected to flotation and differential centrifugations to yield the oil body fraction, the 10,000 × *g* pellet, the 100,000 × *g* pellet, and the 100,000 × *g* supernatant. These subcellular fractions and the total homogenate were subjected to SDS/PAGE, with all loading samples adjusted to represent amounts derived from an equal quantity of the homogenate (Fig. 5). Most of the storage proteins [napin (4 and 13 kDa) and cruciferin (24–30 kDa)] were present in the 100,000 × *g* supernatant, as expected since the storage protein bodies containing these proteins were lysed during the current aqueous isolation procedure (16). Ninety percent or more of the *Brassica* oleosin and the maize oleosin were present in the oil body fraction. Immunoblot analysis of the gel with antibodies raised against the maize oleosin confirmed the almost exclusive localization of the maize oleosin in the oil body fraction (Fig. 5). In the subcellular fractionation, we recovered essentially all the storage proteins in the 100,000 × *g* supernatant, but we recovered only ≈50% of the *Brassica* and maize oleosins in the oil body fraction. This low recovery was due to the difficulties in removing the oil bodies stuck onto the wall of the centrifuge tubes after each centrifugation. The 10,000 × *g* pellet and the 100,000 × *g* pellet contained little protein. When sufficient amounts of the two pellets were subjected to SDS/PAGE, their visible protein patterns resembled that of the total homogenate (data not shown). These proteins might have derived from unbroken and partially broken cells and organelles.

The above localization of the maize oleosin in the *Brassica* oil bodies was not due to an artificial attachment of the hydrophobic oleosin, which might have been present in the cytosol or associated with other cellular membranes, to the oil bodies during tissue homogenization and subcellular fractionation. The evidence comes from the results of the following experiments. Dehulled mature seeds of untransformed *Brassica* plants were homogenized in the grinding medium containing added maize oleosins. Since pure oleosin could be isolated only in the presence of a strong detergent, such as SDS, that would have interfered with the experiment, we used a mixture of maize oleosins (19, 18, and 16 kDa) obtained from isolated oil bodies. Four maize oleosin preparations were used in four experiments: (*i*) oleosins and native phospholipids were obtained in a suspension after the triacylglycerols in isolated maize oil bodies had been extracted with diethyl ether, (*ii*) oleosins alone were obtained after the phospholipids in preparation *i* had been removed by extraction with chloroform/methanol, (*iii*) preparation *i* was sonicated (to generate finer resuspension) and used immediately, and (*iv*) preparation *ii* was sonicated and used immediately. After homogenization of the dehulled seed in the presence of the maize oleosins, the crude extract was subjected to a fractionation scheme identical to that shown in Fig. 5. The four experiments with different maize oleosin preparations yielded very similar results, and the results of the experiment using oleosin of preparation *iii* are shown in Fig. 6. Most of the *Brassica* oleosin (20 kDa) was associated with the oil body fraction, as expected, whereas the maize oleosins (19, 18, and 16 kDa) were present largely in the 10,000 × *g* pellet. The results show that if maize oleosin were associated among themselves or with phospholipids in the cytoplasm in the transformed *Brassica* seed, they would not

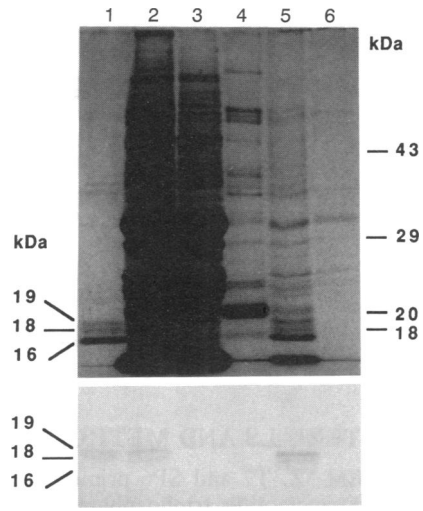

FIG. 6. Separation of seed proteins of nontransformed *B. napus* by SDS/PAGE and immunoblot analysis of the separated proteins. Dehulled mature seed was homogenized in the grinding medium containing added maize oleosins (16, 18, and 19 kDa) and phospholipids, and the homogenate was subjected to fractionation, electrophoresis, and immunodetection as described in Fig. 5. Antibodies raised against the maize 18-kDa oleosin recognized the 18- and 19-kDa oleosins but not the 16-kDa oleosin. (See legend to Fig. 5 and *Results* for other details.) Lanes: 1, maize oil body; 2, total homogenate; 3, 100,000 × *g* supernatant; 4, oil body; 5, 10,000 × *g* pellet; 6, 100,000 × *g* pellet.

have attached to the oil bodies artificially during our homogenization and fractionation procedure.

DISCUSSION

The promoter/terminator of the monocotyledonous maize oleosin gene cannot direct an appreciable expression of the gene in transformed dicotyledonous *Brassica* plants. Since we did not detect the maize oleosin mRNA in the transformants, the absence of the maize oleosin is due not to the instability of the synthesized protein, but rather to the strength of the promoter/terminator in directing transcription or the instability of the mRNA. Other monocotyledonous promoters/terminators have also been shown not to direct the effective expression of genes in dicotyledonous plants (17). In addition, the maize oleosin gene promoter/terminator would not be expected to be strong since the gene product oleosin (of 18 kDa) in the native maize embryo represents ≈1% of the total protein. In contrast, the gene promoter/terminator of the *Brassica* storage protein napin does direct the expression of the maize oleosin gene with correct tissue specificity and temporal regulation. In transformant BP06, the maize oleosin accumulated to 1% of the total seed protein. This high level of expression directed by the specific napin gene promoter/terminator is similar to those of foreign gene expression in seeds directed by other seed storage protein promoters (18, 19).

Since storage proteins and oils share similar accumulation patterns during seed maturation (7), it is reasonable to expect that gene regulatory elements of seed storage proteins could be used in genetic engineering of seed oils. In transformant BP06, the maize oleosin and triacylglycerols accumulated in a similar temporal pattern during seed maturation (data not shown). Our results show that a napin gene promoter/terminator of *Brassica* can be used to express the gene encoding oleosin, which is involved in triacylglycerol accumulation, and suggest that it, or equivalent seed storage protein regulatory elements, may be useful in expression of genes for the genetic engineering of seed oils.

No N-terminal cleavable signal has been found in oleosins (4, 5, 7, 20, 21). When we compare the amino acid sequences and the predicted secondary protein structures of two maize oleosins (4, 5) and one carrot oleosin (6), we recognize that these oleosins are highly similar only in their central hydrophobic domain. This domain would interact effectively with the triacylglycerols in the matrix of the oil body. We hypothesized (4) that the domain serves as a structural anchor of the protein on an oil body and an internal signal for the specific transport of the protein to the oil bodies during organelle synthesis. In the current study, a monocotyledonous oleosin, which shares a close similarity with the dicotyledonous oleosin only at the central hydrophobic domain, is properly synthesized and transported to the oil bodies in transformed *Brassica*. This result supports, but does not validate, the above hypothesis. It is still possible that the targeting signal in the oleosin is only a short amino acid sequence, as in several proteins targeted for the peroxisomes (22) and glycosomes (23); this sequence could be located within or outside the central hydrophobic domain. Identification of the exact location of the targeting sequence should come from transformation studies using oleosins modified by site-directed mutagenesis.

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